VOLUME XVII OCTOBER, 1934 NUMBER 10

JOURNAL OF DAIRY SCIENCE

PUBLISHED BY THE
AMERICAN DAIRY SCIENCE ASSOCIATION

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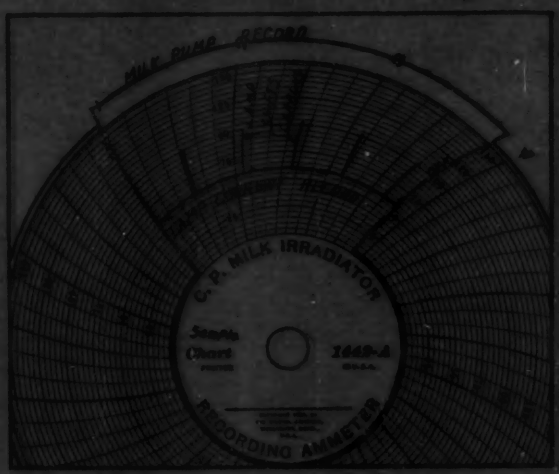

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Entered as second-class mail matter April 13, 1934, at the Post Office at Lancaster, Pa.
Under the Act of March 3, 1879.

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JOURNAL OF DAIRY SCIENCE

VOLUME XVII OCTOBER, 1934 NUMBER 10

THE COLOR IMPARTED TO COFFEE BY CREAM TREATED IN VARIOUS WAYS

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While making a comparison of bottled cream distributed by several competing dairies it was observed that equal volumes of the creams did not always impart to coffee a constant color. This observation, together with reports from consumers that some creams "went further" in coffee than others, led to an investigation of the factors concerned with the color of coffee and cream mixtures.

There are many points which could be included in such an investigation such as the type of coffee used, the manner in which the coffee is prepared and creamed, the influence of temperature of the coffee, a study of the actual shades of brown produced in the coffee-cream mixtures under various conditions, and the influence of the cream itself. However, it is the purpose of this paper to discuss only those factors over which the dairy plant has control, *i.e.*, the cream itself, how its coloring power varies with composition, and the manufacturing processes to which it may be subjected in a bottling plant.

APPARATUS AND METHOD USED TO MEASURE COLORING POWER OF CREAM

The average coffee drinker who flavors and colors coffee usually adds sufficient volume of milk, cream, evaporated milk, or other dairy products to impart a rich golden yellow-brown color to the coffee. To imitate as closely as possible the procedure followed by the average person in creaming coffee, a method of testing cream was developed which determined the volume of any given cream required to impart a constant yellow-brown color to a given volume of coffee of standard strength and temperature.

Preparation of standard coffee solution—One coffee solution was used in all of this work. This was prepared by swirling for 5 minutes in 5 gallons of water at 200° F. a cheese-cloth bag containing 2.2 pounds of medium ground coffee. After the coffee solution had been filtered through a milk filter it was transferred to quart and pint milk bottles. These were filled about two-thirds full and covered with heavy tin foil. The bottles

Received for publication June 28, 1934.

of coffee were then sterilized in an autoclave under 15 pounds of steam pressure for 25 minutes, cooled and stored at room temperature until required.

Color standard—Webb and Holm (1) found the Munsell system of measuring color to be satisfactory in their studies on the color of evaporated milk. By means of rapidly revolving discs of colored paper of selected colors, any given shade of color can be produced. Use was made of this apparatus and three colored discs, yellow, red, and black, in establishing a constant golden yellow-brown standard with which the coffee-cream mixtures could be compared. The shade of color selected was that produced when 1 part of 20 per cent cream is added to 10 parts of average coffee.

How the test was made: A series of large test-tubes holding 100 cc. of the standard coffee solution was prepared. These were heated as required to a temperature of 180–200° F. in a boiling water bath. The coffee was then poured into a small white cup containing 10 cc. of cream at 40–45° F. The coffee-cream mixture was placed beside the revolving color standard and the color compared with that of the standard through an eye-piece assembly which had been removed from a Bausch and Lomb colorimeter of the Duboscq type.

A constant light source was maintained by means of a blue "daylight" frosted lamp installed in such a way that the same amount of light fell at about a 45 degree angle on the surface of both the revolving color standard and the coffee-cream mixture. All other sources of light were carefully eliminated.

The coffee-cream mixture was stirred very gently every few seconds to insure uniformity of color, while a comparison of the colors was being made. Readings were always made in less than 30 seconds to avoid variations due to a decrease in temperature.

If it was found that the color standard was darker than the coffee-cream mixture another test was made using 9.5 cc. of cream. If the color standard was found to be of a lighter shade than the coffee-cream mixture then 10.5 cc. of cream was used in the second test. These tests were repeated using more or less cream as indicated until the color standard was matched.

The number of cc. of cream required to produce the standard color in 100 cc. of coffee, when measured by the method described above, was taken as the coloring power of the cream.

VARIATIONS IN COMMERCIAL PRODUCTS

In order to demonstrate that creams offered to the retail trade vary considerably in their power to impart a given color to coffee, samples of "coffee" cream and "whipping" cream, bottled by thirteen competing

dairies in Baltimore, were purchased retail on the same day. These were examined for acidity, percentage of fat and solids-not-fat, and the amount of cream required to color 100 cc. of coffee. The data are summarized in table 1.

TABLE 1

Variations in cream purchased on the same day from stores and wagons of thirteen dairies competing in the Baltimore area

DAIRY	PURCHASED AS "COFFEE CREAM"				PURCHASED AS "WHIPPING CREAM"			
	Acidity	Fat	Solids-not-fat	Amt. of cream to color 100 cc. coffee	Acidity	Fat	Solids-not-fat	Amt. of cream to color 100 cc. coffee
	%	%	%	cc.	%	%	%	cc.
A	0.16	19.5	8.65	10.4	0.25	24.5	9.20	v. sl. curdled
B	0.11	26.5	6.83	8.9	0.11	36.0	5.56	7.1
C	0.14	20.0	8.12	9.6	0.15	30.0	7.71	8.1
D	0.10	23.0	6.46	9.8	0.09	30.0	9.16	7.9
E	0.10	24.0	6.49	9.7	0.10	32.0	.....	8.1
F	0.11	25.5	7.73	8.9	0.10	29.5	5.39	8.3
G	0.08	21.0	7.69	10.3	0.12	31.5	6.08	8.1
H	0.11	22.0	7.49	10.0	0.10	36.5	4.58	7.4
I	0.11	33.5	6.12	7.7	0.10	33.0	6.67	7.6
J	0.18	20.0	10.41	9.5	0.11	28.5	5.85	8.5
K	0.12	17.5	7.21	10.6	0.10	37.5	5.28	7.1
L	0.24	19.5	13.70	v. sl. crudled	0.15	33.5	8.23	7.1
M	0.16	23.0	7.09	9.7	0.15	34.5	5.70	7.3

It will be seen from the information in table 1 that vast differences exist between products offered to the public by the dairies of Baltimore although bottled and sold under one of two types of cream. Variations of this magnitude would easily be noted by the average coffee drinker in a restaurant where a measured amount of cream is served with coffee and by the more observant consumer who adds cream to coffee from a small pitcher.

Dairies who do not practice close standardization may distribute cream which varies considerably from day to day. This is seen from the data presented in table 2. Dairy C could be depended upon to supply a uniform product, while Dairy B, probably through lack of standardization, was not selling a constant product.

PLANT PRACTICES AND THEIR INFLUENCE ON THE COLOR IMPARTED TO COFFEE

The fat content of the cream. The data presented in table 1 indicate that the fat content of the cream is a factor controlling the color of coffee and cream mixtures. In order to show show the fat content affects the color a series of creams containing various percentages of fat was prepared by standardizing raw 40 per cent cream with skimmilk. These creams were

TABLE 2

Daily variations in "coffee cream" from two competing dairies

DATE 1934 MAY	AMOUNT CREAM REQUIRED TO COLOR 100 CC. COFFEE	
	Dairy B	Dairy C
	cc.	*cc.*
17	9.0	9.7
18	9.0	9.7
19	8.5	9.6
21	9.2	9.6
22	8.9	9.6
25	.....	9.7
26	9.1	9.6
27	9.1	9.7
29	8.6	9.7

pasteurized, cooled and aged one day at 40° F. before testing. The data procured are summarized in table 3.

The milk-solids-not-fat content of creams prepared in this manner varies inversely with the fat content and since it also appears from table 1 that the milk-solids-not-fat has an influence on the color of cream and coffee, another series of creams was prepared as above but with a constant milk-solids-not-fat content. This was accomplished by adding skim condensed milk to the heavier creams. The constant amount of milk-solids-not-fat selected was 7.0 per cent, which is close to the average quantity found in 20 per cent cream. Table 3 also contains the data obtained with this series of cream.

A study of table 3 shows that the color-producing power of cream varies inversely with the fat content of the cream. The coffee drinker therefore requires less of a rich cream than of a thin or low-fat cream.

The milk-solids-not-fat content of the cream. It appears from tables 1 and 3 that the milk-solids-not-fat content of the cream has an influence on

TABLE 3

The influence of fat content of cream on its ability to color coffee

FAT CONTENT OF CREAM	AMT. CREAM TO COLOR 100 CC. COFFEE	
	Normal solids-not-fat in cream	Constant solids-not-fat in cream (7.0%)
%	*cc.*	*cc.*
15	11.1	.....
20	10.0	10.0
25	9.0	9.0
30	8.1	8.0
35	7.0	6.8
40	5.9	5.7

the color imparted to coffee by cream. This inference is substantiated by the data presented in table 4, which gives the observations made on a series

TABLE 4

The influence of milk-solids-not-fat content on ability of 20 per cent cream to color coffee

ADDITIONAL MILK-SOLIDS-NOT-FAT IN CREAM	AMT. CREAM TO COLOR 100 CC. COFFEE	
	Solids from skimmilk powder	Solids from skim condensed milk
%	*cc.*	*cc.*
0.0	10.0	10.0
0.5	9.5	9.6
1.0	9.0	9.1
1.5	8.8	8.8
2.0	8.5	8.6
2.5	8.3	8.3
3.0	8.0	8.1

of 20 per cent creams with increasing milk-solids-not-fat contents. The milk-solids-not-fat content was increased in one case with dry skimmilk and in the other with skim condensed milk. These products were added prior to pasteurization.

Table 4 shows that as the solids-not-fat content of cream is increased its power to color coffee is also increased, so that when cream is fortified with milk-solids-not-fat less cream is required in coffee.

The influence of homogenization. Cream is sometimes homogenized at low pressures to improve the body. That this practice has an influence on the ability of cream to color coffee is shown by the data presented in table 5. This observation confirms that of Mohr and Barfuss-Knochendöppel

TABLE 5

The influence of homogenization on the ability of 20 per cent cream to color coffee

TREATMENT GIVEN CREAM	AMT. CREAM REQUIRED TO COLOR 100 CC. COFFEE
	cc.
Unhomogenized	10.0
Homogenized at 120° F.—	
at 400 lbs. pressure	9.4
at 600 lbs. pressure	9.2
at 800 lbs. pressure	9.1
	(sl. curdled)

(2) who showed that homogenization of 10 per cent and 15 per cent cream increased the "Weiskraft."

When cream is homogenized it appears whiter in color than the same cream unhomogenized (3). The greater the homogenizing pressure the

greater the whitening power of the cream (2). This indicates that the fat globule size is responsible for the difference noted between homogenized and unhomogenized cream.

The influence of pasteurization. A goodly portion of the cream sold in the United States is pasteurized. From table 6 it will be seen that pas-

TABLE 6

The influence of pasteurization on the ability of cream to color coffee

FAT CONTENT OF CREAM	TREATMENT	AMOUNT CREAM REQUIRED TO COLOR 100 CC. COFFEE
%		*cc.*
20	Raw	10.0
	Pasteurized at	
	150° F. for 30 min.	10.0
	185° F. for 15 sec.	9.8
30	Raw	8.2
	Pasteurized at	
	150° F. for 30 min.	8.2
	185° F. for 15 sec.	8.0
40	Raw	6.1
	Pasteurized at	
	145° F. for 30 min.	6.1

teurization at 150° for 30 minutes has no effect on the coloring power of the cream. Flashing cream to 185° F. improved slightly the ability of the cream to color coffee. Mohr and Barfuss-Knochendöppel (2), in addition to making a study of the influence of pasteurization, showed that boiling the cream further increased the whitening power of cream.

By far most of the pasteurized cream sold for table use is treated by the holder method, hence it may be said that pasteurization has no influence on the ability of cream to color coffee.

The influence of aging. Many dairies age cream one or more days after pasteurizing to improve the body of the cream. In order to study the effect of this practice on the coloring power, samples of pasteurized 20 per cent cream were aged for several days at 40° F.

It was found that aging of the cream had no effect on the color imparted to coffee by the cream.

The influence of heat-treating cream. Hening and Dahlberg (4) have shown that when chilled cream is carefully heated to a temperature between 80° and 90° F. and then slowly cooled its viscosity is increased. This may be done either in a vat by the batch method or continuously in tubu-

lar equipment. Table 7 gives the data on two typical creams treated by the batch method.

TABLE 7

The influence of giving cream a temperature treatment to increase its viscosity on ability of cream to color coffee

FAT CONTENT	TREATMENT GIVEN CREAM	AMOUNT CREAM REQUIRED TO COLOR 100 CC. COFFEE
%		cc.
20	Control—normal cream	10.0
20	Heated slowly to 86° F., cooled slowly	10.3
30	Control—normal cream	8.2
30	Heated slowly to 85° F., cooled slowly	8.6

It will be seen from table 7 that when cream is given a temperature treatment to increase its viscosity more cream is required to produce a given color in coffee.

The influence of acidity. Observations on several series of creams of different acidities showed that the acidity of 20 per cent cream is not a factor in determining the quantity of cream required to impart a given color to coffee.

SUMMARY

A test was devised for evaluating creams in regard to the color imparted to coffee.

Bottled creams produced by competing dairies in one city were found to vary considerably in their ability to color coffee.

The amount of cream required to impart to coffee a given color decreased when the fat content increased, when the milk-solids-not-fat content increased, and when the cream was homogenized.

Pasteurization of cream by the usual holding method has no influence on the color of coffee-cream mixtures. Flash pasteurizing seems to have a slight influence.

When cream is given a temperature treatment to increase its viscosity it does not color coffee quite as well as the same cream untreated.

Aging of the cream and the development of slight acidity have no influence on the quantity of cream required to color coffee.

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SOME PHYSICO-CHEMICAL PROPERTIES OF LACTOSE

IV. THE INFLUENCE OF SALTS AND ACIDS UPON THE MUTAROTATION VELOCITY OF LACTOSE

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Lactose contains a free aldehyde group, and therefore it is capable of existing in two different isomeric modifications. When either of these is dissolved in water, it is partially transformed into the other until an equilibrium is reached. The transformation is slow at low temperatures. This fact is of practical importance because the rate of mutarotation sets an upper limit to the rate of solution, or of crystallization of lactose. The fact that the two forms have quite different solubilities is responsible for the recent production of beta lactose on a commercial scale.

Erdmann (11) was the first to observe that the optical rotation of ordinary lactose solutions decreased on standing. He also prepared a new modification of lactose whose optical rotation increased with time. Dubrunfaut (10) also observed the fall in rotation of freshly prepared solutions, and reported that the fall was accelerated by heat. Later, Schmoeger (42) rediscovered what is now known as beta lactose. Although Erdmann's results had been published much earlier, they apparently had not received much recognition (12, 43).

During the latter part of the nineteenth century, a number of papers were published dealing with the mutarotation of sugars. Tanret (46) described three forms of lactose which he called alpha, beta, and gamma lactose. His gamma sugar is now known as beta lactose. The form which he named beta lactose was a mixture of the other two sugars. For a time the equilibrium mixture of mutarotating sugars was quite generally accepted as a distinct modification. Even as late as 1903, we find Roux (41) asserting that the aldoses can exist in three forms.

At the beginning of the twentieth century, several papers appeared which revolutionized ideas regarding mutarotation. Previous to that time, Fischer (13) had suggested that the mutarotation was due to a hydration reaction, and that constant rotation was established only when the sugar was completely hydrated. Trey (47) attributed the mutarotation to some unknown change in configuration. Tanret apparently considered his beta lactose (equilibrium mixture) to be the aldehyde form. Then in 1899, Lowry (25) suggested that the mutarotation was due to the establishment of a dynamic equilibrium between structural isomers, and that the beta

Received for publication March 23, 1934.

form of sugars was not a separate individual but was a mixture of the alpha and gamma forms. Later, after the appearance of a paper by Armstrong (2) showing the relation of the alpha and gamma sugars to the alpha and beta glucosides, Lowry (26) published his theory of dynamic isomerism in more detail. He believed that the change from the alpha to the beta form must be a two stage reaction, and that either the aldehyde form, or its hydrate, must be formed as an intermediate product. He also believed that ". . . apart from its ionizing properties, water is probably directly concerned in the isomeric change of glucose, since this change appears to depend upon a process of reversible hydrolysis." (26, page 1320).

Hudson (16), independently, suggested that the mutarotation of sugars was due to a reversible equilibrium. He published a number of papers dealing with the mutarotation of sugars, particularly of lactose, and discussing the effect of the reversible equilibrium upon the physical behavior of the sugars. (17, 18, 19, 20, 21, 22). Hudson's views differed in one respect from those of Lowry. Lowry postulated an equilibrium between two lactoses with the aldehyde, or its hydrate, as an intermediate, but transitory, form. However, Hudson believed that the mutarotation was due to the reversible reaction,

$$\text{hydrate} = \text{lactone} + \text{water}$$

Hudson believed that hydrated lactose was neither alpha nor beta, but was related equally to each. Lowry (27), however, maintained that the hydrate of glucose was a true hydrate of alpha glucose. More recently, Gillis (14) has claimed also that hydrated lactose is a hydrate of the alpha modification.

In recent years, there has been considerable speculation regarding the actual mechanics of the mutarotation reaction. There is no question about the nature of the end products. The lactone formula is generally accepted, for the alpha and beta sugars, but there is some argument about the nature of the intermediate compounds if such exist. The evidence is largely indirect since it is based chiefly upon a study of the factors which influence the velocity of mutarotation.

Many papers have appeared showing that the mutarotation of lactose, or of sugars in general, is influenced by many factors (51, 52, 44, 45, 47, 48, 38, 49, 26, 27, 19, 37, 1, 33, 50, 39). In general, the mutarotation of sugars is accelerated by an increase in temperature. It is also accelerated by the action of alkalies and acids. Alkalies are more effective than acids, consequently the minimum velocity of mutarotation is not at true neutrality but at pHs below seven. Mutarotation is retarded by alcohols, and by acetone. The action of salts is variable. Some salts have been reported as influencing the mutarotation velocities, but this is commonly attributed to hydrolysis, with a consequent change in pH.

Regarding the mechanics of mutarotation, there are two principal theories. In order to convert alpha lactose to beta lactose, it is necessary only that there be a migration of one proton. Baker believed that this migration might take place directly, without the intervention of any other substance. He has published several papers in support of this theory. (3, 4, 5, 6). Opposed to this view, we have the statement of Lowry, "Catalysis by acids and bases of prototropic changes and of hydraulysis, is interpreted in terms of electrolytic theory according to which a flow of valency electrons through the molecule is produced by bringing a proton donator, and a proton acceptor or a hydroxyl donator, into contact with the two terminals of the labile complex." (30, page 2565). In an earlier paper, he had asserted that, "There is no valid evidence in the literature to prove that mutarotation can take place except by the intervention of water." (28, page 1375). In subsequent papers, this idea was extended as is indicated by the first quotation. Briefly, it is believed that the transfer of a proton is brought about in two or more stages. One proton is withdrawn from one part of the molecule, while another proton is supplied to some other part, thereby keeping the molecule electrically neutral. In a later stage, this process is reversed, but the new proton does not necessarily enter into the same position which was originally occupied by the proton which was withdrawn. In this way, the transformation from one isomer to the other is accomplished. According to this theory, the mutarotation of sugars can occur only in the presence of a substance capable of supplying protons to the molecule and a substance capable of accepting a proton from it. Both a donator and an acceptor must be present. In recent years, largely as the result of studies of systems in non-aqueous solvents, many substances have been found to serve as donators and acceptors of protons. (9, 8, 24, 15). Accordingly, many substances might influence the mutarotation of sugars. This theory that mutarotation can occur only in the presence of a donator and an acceptor of protons has been developed by Lowry (28, 23, 29, 30, 31, 32, 34, 40, 35).

The possibility that a variety of substances might accelerate the mutarotation of lactose seemed worthy of study because the mutarotation velocity of lactose is a factor governing the maximum velocity of crystallization, and of solution. If mutarotation is accelerated greatly by substances normally present in dairy products, then these calculations are not valid. For this reason, an attempt was made to secure data which would indicate the magnitude of the errors which might be introduced by the action of the various milk constituents. At the same time, it was resolved to review, critically, the published data regarding the variation of mutarotation velocity with changes in pH.

EXPERIMENTS

Measurements of the optical rotations of sugar solutions were made by means of a Schmidt and Haensch polariscope which could be read to 0.01°. The polariscope tube was 400 mm. in length. It was provided with a jacket through which water was circulated from a large thermostat. The thermostat was maintained at 25.0° C., but the temperature of the solutions was often slightly higher than this, due to absorption of heat from the surroundings. However, in any given run, the temperature was usually constant, and was determined by immersing a thermometer in the contents of the tube. The illumination was provided by a monochromator. Unfortunately, this instrument was out of adjustment, and the exact wave-length in use could not be ascertained. For this reason, the values found for the optical rotation of various solutions are not comparable with the data of others.

Even though the wave-length of the light was unknown, the fact that it remained constant during a series of experiments, made it possible to determine the velocity of mutarotation. It was also possible to determine the effect of various added substances upon the equilibrium rotation of lactose. Accordingly, the work reported in this, and in the following section, was limited to a study of these two phenomena.

If catalytic activity is a general property of proton donators and acceptors, then catalytic action might be expected from not only the hydrogen and hydroxyl ions, but also from the undissociated molecules of acids and bases, from the anions of weak acids, and the cations of weak bases.

It is well known that the velocity of mutarotation of lactose may be greatly altered by changes in the pH of its solutions. This effect will be discussed in more detail, later. At this time, it is sufficient to state that the velocity of mutarotation is practically constant through the pH range from three to six. In order that slight changes in pH might not obscure the effects of other catalysts, all experiments reported in this section were carried out at pHs between 4.0 and 5.0.

The velocity of mutarotation was determined by placing 5.00 grams of recrystallized alpha hydrate in a 100 cc. volumetric flask. This sugar was then dissolved in the solvent (water or salt solutions) and the flask was filled to the mark. After mixing thoroughly, the solution was transferred to the polariscope tube. A series of observations was then made at ten minute intervals from which the mutarotation velocity could be calculated. In order to increase the accuracy of these observations, each value was determined by making a series of readings in rapid succession, beginning shortly before the ten minute interval was ended. Later, by plotting these readings against time the most probable value of the rotation at the exact moment selected could be determined with considerable accuracy. The

rotation at equilibrium was determined after the solution had remained at approximately twenty-five degrees for twenty-four hours. The mutarotation constant, $k_1 + k_2$, was determined by means of the equation,

$$k_1 + k_2 = \frac{1}{t} \log \frac{V_o - V}{V_t - V}$$

In this equation, V is the equilibrium rotation, V_t is the rotation at a time t hours after the initial observation, V_o, was made. Logarithms to the base ten were used.

Using this method, the mutarotation velocity of lactose was determined in pure water, and in solutions of several salts. For these experiments, a normal solution of ammonium chloride was used to test the influence of a cation of a weak base, potassium acetate was used to test the influence of an anion of a weak acid, and ammonium acetate was used to test the combined influence of the two ions. As a control, the mutarotation of lactose was determined in pure water, and in solutions of potassium chloride, which was chosen as a representative of the salts of strong acids and strong bases. In each case, the solvent was acidified with HCl to a pH of approximately 5.0, as estimated by means of methyl red. The data are shown in Table 1.

TABLE 1

The mutarotation velocity of lactose in normal salt solutions

SALT USED	TEMPERATURE	$k_1 + k_2$
None	25.3° C.	0.475
Potassium chloride	25.0	0.413
Ammonium chloride	25.4	0.501
Potassium acetate	25.0	2.8
Ammonium acetate	25.0	3.5
Potassium chloride	25.0	0.404

All solutions were adjusted by means of hydrochloric acid and methyl red to a pH of approximately 5.0.

The results of these experiments indicate a marked catalysis of the mutarotation of lactose by the anions of weak acids. A normal solution of potassium acetate is more effective as a catalyst than a tenth normal solution of hydrogen ions, Table 2. On the other hand, though the anion of the weak base, ammonium hydroxide, exhibits a definite effect, it is very small compared with the effect of the acetate ion, even though the dissociation constants of acetic acid and of ammonium hydroxide are practically the same. This is in agreement with the fact that the hydrogen ion, a

TABLE 2

Calculated values of the mutarotation constant of lactose at various values of pH. Temperature 25° C.

pH	k_1+k_2	pH	k_1+k_2
0.0	19.5	5.0	0.46
0.5	4.7	6.0	0.48
1.0	1.74	6.5	0.54
1.5	0.94	7.0	0.68
2.0	0.65	7.5	1.04
2.5	0.52	8.0	2.07
3.0	0.48	8.5	5.9
4.0	0.46	9.0	27.6

These values are based on the data of Troy and Sharp (50).

proton donator, shows much less catalytic effect than the hydroxyl ion, a proton acceptor.

The action of potassium chloride seems anomalous, since it seems to retard the mutarotation of lactose. No explanation of this phenomenon has been found. At first, experimental error was suspected, but a repetition of the experiment with new reagents gave the same result. Finally, a search of the literature revealed that this phenomenon had been observed by others. Lowry (26) reported in 1903 that the mutarotation of glucose was retarded in normal solutions of potassium chloride. Mukhin and Ass (37) found, in 1925, that 0.5 normal potassium chloride reduced the mutarotation velocity of glucose by 6.1 per cent, and that 0.5 normal sodium chloride reduced it by 3.4 per cent. On the other hand, in 1903, Trey (49) reported that sodium chloride had no effect upon the mutarotation of glucose and Andrews and Worley (1) also reported that pure sodium chloride, in concentrations as high as 8.6 mols per thousand mols of water, had no effect.

If we accept the fact that, at high concentrations, KCl does retard the mutarotation of lactose, it becomes a matter of some interest as to whether this effect is due to the undissociated salt, to the potassium ion, or to the chloride ion. This matter has not yet been investigated.

As early as 1897, Trey (48, 49) reported that the mutarotation of lactose was accelerated by many salts. Similar effects have been observed by others with glucose. Trey believed that hydraulysis, with the consequent liberation of acid or of alkali, was responsible for this phenomenon. To what extent pH changes may account for his results cannot be determined at present. Mukhin and Ass believed that the phenomenon was due to the formation of compounds in solution which had a greater mutarotation velocity in solution than the free sugars. The results obtained in this investigation seem to support the theories of Lowry, and of Brönsted,

regarding acid and basic catalysis. However, it is quite possible that other factors might influence mutarotation velocities. Certainly a satisfactory theory must account for the retarded mutarotation of lactose in concentrated solutions of potassium chloride.

After having demonstrated that the mutarotation velocity of lactose was influenced by ions other than hydrogen and hydroxyl, it became desirable to determine the actual mutarotation velocity of lactose in milk, and in the various dairy products. Direct determination was not possible, however, because of the turbidity of such solutions. Until other means of determination could be developed, it was necessary to work with simple solutions. So far this part of the investigation has been limited to a study of the effect of lactates and lactic acid at various concentrations. Lactates were chosen as typical salts of weak acids. It is true that lactates are not found in sweet milk products, but other salts of weak acids are found there.

A lactate buffer solution was prepared from C.P. sodium carbonate and C.P. lactic acid. The solution had a pH of approximately 4.3. It was boiled for 15 minutes to expel all carbon dioxide. This solution contained one molecular weight of sodium lactate per liter, plus an excess of lactic acid. From this original solution, three solutions were prepared, by dilution, having one-tenth, one-hundredth, and one-thousandth the concentration of the original solution. Although the concentration of the undissociated acid, and of the lactate ion, in these solutions, varied through a range of 1 to 1000, the concentration of hydrogen ions was practically the same in each solution. Actually, the variation in hydrogen-ion concentration was only in the ratio of 1 to 2.3, and this variation is too small to cause any perceptible change in the velocity of mutarotation at the pH of these solutions.

The mutarotation velocity of lactose solutions (5 grams per 100 cc.) was determined in these four solutions which had practically the same pH, but different concentrations of salt and acid. The data which were obtained are recorded in Table 3.

It was necessary to make a small correction to the observed rotations of these solutions because the lactic acid used for the buffer was slightly dextro-rotatory. This correction, amounting to 1.12° in the case of the most concentrated buffer solution, was subtracted from all of the observations before the calculations of mutarotation velocity were made.

From the data in Table 3, it seems probable that the acid and salt in the most dilute buffer had no measurable influence upon the mutarotation velocity of the lactose. When the salt concentration was one-hundredth normal, the velocity increased slightly. At concentrations of tenth normal, the mutarotation constant was increased about 40 per cent. At higher concentrations, the influence of the salt and acid increased enor-

TABLE 3

The mutarotation constant of lactose in lactate buffer solutions of different concentrations. Temperature, 25° C.

CONCENTRATION OF SODIUM LACTATE	pH	k_1+k_2		
		First trial	Second trial	Average
Normal	4.32	1.74 1.79 1.74	1.76 1.78 1.84	
		(1.76)	(1.79)	1.77
0.1 normal	4.32	0.625 0.612 0.627	0.675 0.643	
		(0.621)	(0.659)	0.636
0.01 normal	4.44	0.542 0.511 0.517	0.601 0.487 0.515	
		(0.525)	(0.534)	0.529
0.001 normal	4.68	0.500 0.478 0.454	0.451 0.471 0.480	
		(0.477)	(0.467)	0.472

mously. This is analogous to the effects of the hydrogen, or hydroxyl ions, whose catalytic power increases slowly at first, and then more rapidly as the concentration is increased.

If we attempt to apply these data to dairy products, it becomes apparent that lactic acid will seldom accelerate the mutarotation of lactose. Milk is coagulated by approximately 0.45 per cent of lactic acid. This is equivalent to only 0.05 normal, and at the resulting pH of 4.7±, a mutarotation constant of approximately 0.57 might be expected. This, however, is less than the value of the constant at a pH of 6.6–6.7 (the pH of fresh milk) in the absence of lactic acid.

At present, data regarding the effect of other proton donators and acceptors in milk are not available. However, if the catalytic action of other ions and molecules changes as rapidly with change of concentration as is true of lactates and lactic acid, then their action is probably of minor importance in unconcentrated products, but may be very important in such concentrated products as ice cream and milk powders. However, more data are necessary, particularly with respect to the catalytic action of proteins, before definite conclusions may be drawn.

Since molecules and ions other than hydrogen and hydroxyl influence the mutarotation velocity of lactose, it becomes necessary to examine the

data regarding the effect of pH upon this, to determine how greatly they might be in error due to interference by accelerating agents whose influence had not been recognized. The influence of acids and alkalies upon the mutarotation velocity of lactose has been shown by a number of workers (51, 52, 48, 49, 7, 50, 39). Of these, the last two have published curves showing the values of the mutarotation constant at various values of pH.

At first glance, the curves of Troy and Sharp (50) and those of Parisi (39) seem to differ considerably. Most of the difference, however, seems to arise from the fact that Parisi did not study the behavior of solutions of pH less than 1.6, and did not observe the marked acceleration of the mutarotation velocity which occurs below pH 1.0, a phenomenon known since 1882 (51).

We may examine the technique used in the two researches to estimate the likelihood of errors introduced by interfering catalysts. Parisi used unbuffered solutions. Troy and Sharp used very dilute buffers. The method of Parisi should be practically free from errors due to catalysts other than hydrogen and hydroxyl. The use of buffers is subject to criticism, but the buffer solutions used by Troy and Sharp were so dilute that the errors involved are practically negligible.

Parisi's paper contains some equations which were derived to show the relation between the values of k_2, of temperature, and of pH. Others have attempted to write equations for the relation of $k_1 + k_2$ to the pH, but Parisi is the first to attempt to include temperature as one of the variables. In doing so, he introduced an error. In his derivation he substituted $K_w = 10^{-14}$, a substitution which is only valid at approximately 25° C. Because of variation in K_w (36), the constant 1.743×10^{12} of Parisi's equation should become 1.61×10^{12} at 40° C. and 1.79×10^{12} at 16° C.

Parisi's attempt to derive an equation relating the mutarotation constant to the pH suggested that the data of Troy and Sharp might serve as the basis for such an equation. An empirical equation was therefore derived which gives values agreeing very well with those determined by experiment. This equation may be written in the form,

$$\log\ (k_1 + k_2) = 0.00415\ (pH - 4.45)^4 - 0.34$$

Using this equation, the values of the mutarotation constant were calculated for various values of pH. They are given in Table 2. These values are, of course, only applicable at temperatures of 25° C., and to solutions free from appreciable amounts of other catalytic substances.

SUMMARY

The mutarotation of lactose is accelerated by molecules, and by ions other than those of hydrogen and hydroxyl. This phenomenon is attributed to general acid and base catalysis.

The catalytic influence of the anions of weak acids is much greater than that of the cations of weak bases.

The catalytic effect of the lactate ion is relatively small at concentrations below one-tenth normal, but it increases very rapidly as the concentration becomes greater.

The catalytic effects of other salts found in dairy products have not yet been determined, but it seems probable that their influence would not be important, except possibly in such concentrated products as milk powder and ice cream.

An empirical equation, based upon the data of Troy and Sharp, has been derived for estimating the velocity of mutarotation of lactose solutions at 25° C., and at various values of pH.

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LESPEDEZA STRAW FOR DAIRY COWS

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The palatability and value for milk production of lespedeza straw were studied in a feeding trial with dairy cows. Comparison was made with soybean hay, chosen because of the fact that both lespedeza and soybeans, unlike most other legume crops used in feeding dairy cattle, can be grown on acid soils.

A number of studies of the feeding value of lespedeza hay have been made. Moore (1) reports "very little difference in the feed value of these legumes (alfalfa, lespedeza and soybean hays) when all are cut at the right state and properly cured."

Moore and Cowsert (2) reported that dairy cows produced slightly less milk but gained more weight when fed lespedeza hay than when soybean hay was fed. In a comparison (2) of lespedeza and alfalfa hays for milk production, it was found that less feed was required to produce a unit of product when alfalfa was given.

The North Carolina Station (3) reported a slight advantage of alfalfa hay over lespedeza hay for dairy cattle.

The Tennessee Station (4) reported that *Sericea lespedeza* hay, carrying about 25 per cent of crab grass, gave better results with yearling Jersey heifers than grass hay, but the gains were not equal to those made with alfalfa.

The author has been unable to locate in the literature any reports of experimental investigations of the feeding value of lespedeza straw for dairy cattle.

DESCRIPTION OF FEEDS USED IN TRIAL

The *lespedeza straw* came from Korean lespedeza grown for seed in Crawford County, Illinois. The crop was harvested before complete maturity of the seed and was threshed and baled without exposure to rain. The leaves were saved carefully. Approximately 65 per cent of the straw consisted of leaves, 27 per cent of stems and 8 per cent of foreign matter composed principally of timothy stubble. At the time of feeding, part of the leaves were green and part light brown in color. The stems were dark brown or brownish red. The hay was free from mold and mustiness and was in good condition for feeding. The stems were hard and somewhat wiry.

The *soybean hay* was grown on the University Farm and was harvested when the pods were forming. The pods were almost devoid of seed. The

Received for publication June 22, 1934.

hay was cured without exposure to rain, baled in the field and stored at once. The hay was bright green in color and was free from mold, musty odors and excessive dust. Portions of several bales were taken during the feeding trial and the sample thus obtained separated into pod, stem, and leaf (entire leaf including petiole) portions. The pods comprised about 5 per cent, the stems 31 per cent, and the leaves 64 per cent of the sample.

The *corn silage* was of good quality. It was made from heavily-eared Reid Yellow Dent Corn, well advanced in maturity. It contained about 32 per cent dry matter.

The *grain mixture* was made up according to the following formula: shelled corn, ground, 425 pounds; oats, ground, 200 pounds; wheat bran, 250 pounds; soybean oil meal, 100 pounds; salt, 15 pounds; steamed bone meal, 10 pounds. It contained approximately 14 per cent total protein.

GENERAL PLAN OF FEEDING TRIAL

The trial consisted of two periods, each four weeks in length. Period I was preceded by a 2-weeks' preliminary period and Period II by a 1-week transition period.

Two groups of cows were used. Each group consisted of eight Holsteins and one Ayrshire. The groups were made up by choosing two cows as nearly alike as possible with respect to stage of lactation, milk yield, etc., and placing one cow in Group I and the other in Group II.

The Holsteins were fed 30 pounds of silage per head daily, and the Ayrshires 20 pounds. Toward the close of the trial, it was necessary to reduce the amounts fed to some of the cows on account of a higher acid condition in the silage as the bottom of the silo was neared.

The hay and straw were fed in amounts such that each group consumed approximately the same number of pounds. The soybean hay, however, was limited to amounts which would be eaten without waste of leaves, pods and fine portions of the stems. Only the coarse stems remained. It was not possible to adjust the feeding of lespedeza straw so closely, because the cows did not make a clean separation of the leaves from the stems. The refused portions of the hay and straw were collected from the mangers frequently and placed in large gunny sacks. Weekly records were made of the refusal of each cow. The refused portions, which absorbed moisture as a result of being nosed over by the cows, were dried and the amounts calculated to the same moisture basis as the hay fed. Samples of all feeds for analysis were taken regularly.

At the close of Period I, the group of cows which had been fed lespedeza straw was fed soybean hay and the group which had received soybean hay during Period I was given lespedeza straw.

Each milking of each cow was weighed and sampled. Composite samples were tested weekly for butterfat content. The cows were weighed on

three consecutive days at the beginning and end of each period and also once weekly.

COMPARATIVE FEEDING VALUES

Lespedeza straw was not as palatable as the soybean hay. There was, however, considerable variation in the preferences of the cows. Eight of the cows consumed as much of the lespedeza straw as of the soybean hay.

A true picture of the relative palatability of the two feeds is not given by the figures shown in Tables 1 and 2, because the amounts of soybean hay fed to a number of cows were limited in order to keep the dry roughage con-

TABLE 1

Summary of results of feeding lespedeza straw in comparison with soybean hay

KIND OF FEED	NO. OF COWS	FEED CONSUMED DAILY PER COW			GAIN IN WEIGHT DAILY PER COW	TEST OF MILK	MILK YIELD DAILY PER COW[1]
		Silage	Straw or hay	Grain			
		lbs.	*lbs.*	*lbs.*	*lbs.*	*%*	*lbs.*
Lespedeza straw	18	28.4	12.0	12.8	.37	3.71	33.5
Soybean hay	18	28.4	12.8	12.8	.08	3.78	35.5

[1] Milk energy in terms of 4% milk computed according to the formula .4 × milk (in pounds) + 15 × fat (in pounds).

TABLE 2

Proportions of straw and hay refused

KIND OF FEED	NO. OF COWS	AMOUNT FED DAILY PER COW	AMOUNT REFUSED DAILY PER COW		AMOUNT CONSUMED DAILY PER COW
		lbs.	*lbs.*	*%*	*lbs.*
Lespedeza straw	18	13.3	1.3	10.0	12.0
Soybean hay	18	16.6	3.8	23.1	12.8

sumption of the two groups of cows approximately the same. Probably more soybean hay would have been consumed had it been offered, but this would not have been likely for the lespedeza straw except in the case of one cow, because all cows in the lespedeza group were fed to the limit of appetite except for this one cow.

Milk production was slightly less while the cows were fed lespedeza straw than when they received soybean hay, but gains in weight were slightly greater when the cows were fed the lespedeza (Table 1). Ten per cent of the lespedeza straw fed was refused while 23 per cent of the soybean hay was refused (Table 2).

The lespedeza straw was much lower in protein and somewhat higher in fiber content than the soybean hay (Table 3). The refused portions of the

TABLE 3

Composition of lespedeza straw and soybean hay

KIND OF ROUGHAGE	DRY MATTER	ASH	TOTAL PROTEIN	FIBER	ETHER EXTRACT	N-FREE EXTRACT
	%	%	%	%	%	%
Soybean hay	90	8.0	12.6	25.9	1.5	42.0
Lespedeza straw	90	4.6	6.8	29.2	2.3	47.1
Soybean stems[1]	90	3.5	7.7	43.1	.9	34.8
Lespedeza stems[1]	90	3.6	5.1	36.6	1.3	43.4

[1] Portions of roughage refused by cows during feeding trial.

straw and hay were lower in protein and higher in fiber than the feeds from which they came, indicating that the refuse consisted of the coarser portions of the feeds.

SUMMARY

Lespedeza straw consisting of the roughage from a crop of lespedeza harvested before maturity and threshed for seed, proved somewhat less palatable and slightly less valuable as a feed for dairy cows in milk than soybean hay harvested in the early stages of pod formation.

Twenty-three per cent of the soybean hay fed was refused while only ten per cent of the lespedeza straw was refused.

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FAT RISING IN CREAM[1]

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A study of the extent to which butterfat tends to rise in cream was undertaken primarily to seek an explanation for the distribution of butterfat in frozen cream. Moreover, the dearth of literature pertaining to the rise of butterfat in cream required that a more complete study be made in order to understand better the effect of the factors involved.

The abundance of literature on the rise of butterfat in milk offers a good fund of information concerning the phenomenon of fat rising. The fundamental cause of cream rising is the difference between the specific gravity of butterfat and the plasma in which the fat globules are dispersed. Various investigators have attributed the differences in the rapidity and completeness with which the fat rises in milk to the size of the fat globules and the degree of their association, the plasma-solids content of the milk, heat treatments to which milk is subjected, viscosity, individuality of the animal producing the fat, electric charges on the fat globules, and agitation of the milk.

Evidence which gave definite data concerning the distribution of fat in cream was found in the paper reported by McInerney and Sharp (4) on fat distribution in gravity cream. Their experimental results showed that the fat content of the cream layer in milk bottles decreased progressively from the top to the bottom. Fundamental evidence supporting this work was presented by Troy and Sharp (9) in their rather complete study of the physical factors involved in fat rising of milk. Dahlberg and Marquardt (1) have also secured analyses of fractional parts of gravity cream which indicate that the lower fraction tests less than the top portion. A progressive decrease in the fat content of the skimmilk below the cream line was found by McInerney and Sharp, and Dahlberg and Marquardt. However, these studies were made on gravity creams which were produced under entirely different conditions than those separated centrifugally. While these investigators demonstrated the possibility of a range in the fat content from the top to the bottom of cream, this cannot be taken as evidence that such is always the case, particularly with mechanically separated creams containing higher percentages of butterfat.

Received for publication May 25, 1934.

[1] The data upon which this paper is based were taken from a thesis presented by H. C. Trelogan in partial fulfillment of the requirements for the degree of M.S., University of Minnesota.

Paper No. 1272, Journal series, Minnesota Agricultural Experiment Station.

EXPERIMENTAL METHODS

Three rather distinct types of cream were used for these studies, *i.e.*, low fat cream containing near 20 per cent butterfat, medium fat cream with about 30 per cent butterfat and high testing cream containing approximately 40 per cent fat.

Methods somewhat similar to those employed by Palmer and Anderson (6) in their estimations of cream rising in milk were used for this experiment. Cream was placed in 200 milliliter graduated cylinders, in the bottom of which holes 3/16 inch in diameter had been bored so that fractional parts of the cream could be drawn off separately with a minimum amount of mixing taking place. Small wooden plugs were used to stop the holes until such time as the holding period was over when the stopper was removed and the cream permitted to flow out slowly.

Samples from the lower, middle and top fractions of the cream were drawn off separately after the cylinder had been permitted to stand the required length of time. The sample representing the lower fraction was the bottom 20 milliliters and the top fraction consisted of the last 20 milliliters taken out of the cylinder, it being the upper tenth portion of the cream. These samples were kept separate and tested individually so that a comparison of the tests could be used to ascertain the degree of fat rising which had occurred in the cream that was held in the cylinder.

For each cream study made, holding periods of 6, 12, 18, and 24 hours were maintained. This was accomplished by mixing the cream thoroughly immediately after it was standardized to insure a uniform distribution of the fat. Five cylinders were then filled with the mixed cream. The cylinders were all placed on a rack which was put into a cooler where it would not be disturbed. At the end of six hours the first cylinder was removed from the rack and fractionated. The second cylinder was removed after 12 hours, the third after 18 hours, and so on. In case any of the first four cylinders were not correctly sampled, the fifth cylinder was used in its stead, but when no accidents occurred, the fifth cylinder was used for observing the progress of the fat rising after a longer holding period.

Raw Cream

Six trials were conducted with raw cream which represented the three different fat contents. In some cases the cream was standardized to the required fat percentage. The cream was cooled to 40° F. before it was placed in the cylinders and the temperature of the cooler was maintained below that temperature and above freezing at all times. The data presented in table 1 represent the average results for the six trials.

The average figures show that the distribution of fat in high-testing creams was fairly uniform even after they had been allowed to stand for 24 hours. Nevertheless, a slow gradual rise of the butterfat appeared to

TABLE 1

Average butterfat tests of top, middle, and bottom fractions of raw cream held below 40 degrees F.

TYPE OF CREAM	FRACTION	BUTTERFAT CONTENT AFTER			
		6 hours	12 hours	18 hours	24 hours
		per cent	*per cent*	*per cent*	*per cent*
High fat	Top	39.58	39.71	39.75	40.17
	Middle	39.42	39.50	39.58	39.58
	Bottom	39.42	39.21	39.37	39.17
Medium fat	Top	30.21	30.71	31.33	31.42
	Middle	30.21	30.27	30.21	30.21
	Bottom	30.33	30.08	29.92	29.81
Low fat	Top	20.92	21.75	24.50	23.75
	Middle	20.10	20.14	20.25	20.33
	Bottom	18.17	17.00	15.67	15.00

take place, which rise, at the end of a day, caused the top portion to test almost one per cent higher than the bottom portion. Although these are average results, in no case did the top sample test less than 0.5 per cent more than the bottom fraction. The maximum difference between the top and bottom fractions was 1.5 per cent of fat. No greater differences were found when a two-day holding period was used. The fat rise in this type of cream proved to be slow but definite at temperatures below 40° F.

In the cream of medium fat content the fat rise was somewhat greater inasmuch as the differences between the top and bottom fractions were on the average about twice as large as in the high fat creams. When this type of cream was permitted to stand for periods longer than one day, there was a continued slow rising of the fat.

The low-fat cream was subject to far more fat rising than the other two types. The average results show that in the course of six hours the fat rose to a greater extent than it did in the medium fat cream during 24 hours. At the end of one day the difference in tests between the top and bottom fractions was at least 6.5 per cent and the average for all the trials was more than 8 per cent. The rise of the fat in this type of cream continued to be very marked beyond 24 hours and in one sample held for 168 hours the top portion tested 34 per cent while the bottom portion contained less than 9.5 per cent fat.

In every trial the test of the middle portion remained practically the same as the original test of the cream. The loss of fat to the upper part was probably compensated by the gain from the lower portion. However, it was particularly noticeable in the low fat creams that the fat was more likely to leave the bottom fraction than it was to concentrate in the top por-

tion. This resulted in a tendency for a middle portion to test slightly more as the holding periods became longer. The increase in the fat content of the middle portion was evidently small because this fraction was eight times as large as the other fractions.

Holding Temperatures

Single trials were conducted with the three types of cream held at higher temperatures in order to demonstrate the influence that holding temperatures may exert on the fat rising. In one case, the creams were placed in a room maintained at 54° F.; and in the other, the creams were kept in a room at prevailing summer temperatures, namely, for the high fat cream, 84° F.; for the medium fat cream, 82° F.; and for the low testing cream, 76° F. There was a maximum variation of 1° F. in these holding temperatures. One per cent of formalin was added to the cream to prevent

TABLE 2

Butterfat content of top, middle, and bottom fractions of raw cream held at 54° F. and at room temperature

TYPE OF CREAM	FRACTION	BUTTERFAT CONTENT AFTER			
		6 hours	12 hours	18 hours	24 hours
		per cent	*per cent*	*per cent*	*per cent*
		Held at 54° F.			
High fat	Top	39.0	38.5	39.5	40.0
	Middle	38.5	38.5	38.5	38.5
	Bottom	38.5	38.5	38.0	38.0
Medium fat	Top	29.0	30.0	31.5	32.0
	Middle	29.0	29.0	29.0	29.0
	Bottom	28.0	27.0	25.5	26.5
Low fat	Top	21.0	22.5	25.5	26.0
	Middle	20.5	20.5	20.5	20.5
	Bottom	18.0	16.0	14.0	14.5
		Held at Room Temperatures			
High fat	Top	41.5	41.0	42.0	42.0
	Middle	39.5	39.5	39.5	39.5
	Bottom		39.5	39.5	39.0
Medium fat	Top	33.5	35.0	36.0	36.0
	Middle	31.0	30.7	30.6	30.7
	Bottom	30.5	30.0	27.5	25.0
Low fat	Top	25.2	[1]	31.0	33.0
	Middle	20.6		20.5	20.5
	Bottom	17.5		10.0	7.0

[1] Samples discarded.

souring and curdling which would form a mechanical obstruction, thus preventing fat from rising.

The results of these trials, presented in table 2, indicate that holding temperatures have a decided influence upon the extent to which fat rises in cream. The most pronounced differences were evident in the low testing cream, the bottom tenth of which contained only 7 per cent of butterfat after holding for 24 hours at room temperature. The high fat cream was least affected, nevertheless, the influence was decided, as there was a three per cent difference between the top and bottom portions at the high temperature. In fact the difference between the tests of the top and bottom samples was approximately three times greater at room temperature than at 40° F. in all three types of cream.

Obviously, these holding temperatures were distinctly higher than would be expected in practice for such long periods of time. Nevertheless, the tendency of high temperatures to aid fat rising, and low temperatures to hinder it, was illustrated.

Pasteurized Creams

Cream of all three types was pasteurized at 150° F. for 30 minutes after which it was cooled and placed in the graduated cylinders. Four times this was tried with each type and the cylinders were held below 40° F. The average results of these trials appear in table 3.

TABLE 3

Average butterfat content of top, middle, and bottom portions of pasteurized creams held below 40° F.

TYPE OF CREAM	FRACTION	BUTTERFAT CONTENT AFTER			
		6 hours	12 hours	18 hours	24 hours
		per cent	*per cent*	*per cent*	*per cent*
High fat	Top	41.50	41.38	41.87	42.00
	Middle	41.06	41.00	41.00	41.06
	Bottom	40.75	40.75	40.62	40.75
Medium fat	Top	30.12	30.37	31.37	31.88
	Middle	29.69	29.81	29.87	29.62
	Bottom	29.00	28.75	28.12	28.31
Low fat	Top	21.83	23.33	25.83	26.00
	Middle	20.87	21.25	21.33	21.50
	Bottom	16.00	14.33	12.83	12.50

Using the average figures of the raw cream trials as the basis for comparison, the data for pasteurized cream show the same general trend in the results obtained, but it is apparent that the fat tends to rise to a greater extent in the pasteurized cream. It is interesting to note that one sample of

medium fat cream was held for a period of three weeks, after which the top fraction tested 49.5 per cent, the bottom fraction less than one per cent and the test of the middle portion increased one per cent.

Pasteurized Milk

Palmer, Hening, and Anderson (6) secured results indicating that the effects of heat on cream rising were due to the effects of heat on the milk plasma. Therefore, it was planned to pasteurize a quantity of milk and separate the cream to note if there were any differences in the fat rising which took place in the resulting cream.

Eight gallons of milk obtained from the University herd were pasteurized in a hot water bath at 150° F. for 30 minutes, then cooled at 90° F. and separated. A cream of medium fat content was secured and cooled at 40° F. for the fat rising study. The cylinders were filled and held in a cooler maintained at 35° F. The results are presented in table 4.

TABLE 4

Butterfat content of top, middle, and bottom portions of cream from pasteurized milk

FRACTION	BUTTERFAT CONTENT AFTER				
	6 hours	12 hours	18 hours	24 hours	74 hours
	per cent fat	*per cent fat*	*per cent fat*	*per cent fat*	*per cent fat*
Top	30.5	30.5	31.0	31.25	32.25
Middle	30.5	30.5	29.75	30.25	30.50
Bottom	30.5	30.5	30.5	30.50	27.75

Judging from these data, the fat rising seems to be delayed in cream separated from pasteurized milk for there was no apparent rise until after 12 hours of holding. At the end of one day there was less difference between the top and bottom tests of this cream than there was in the other pasteurized cream trials.

Standardization

The effect of standardization was studied by separating one batch of cream to approximately 35 per cent butterfat and another to 46 per cent. The 46 per cent cream was then standardized with skim-milk to nearly the same fat content. These creams were placed in the graduated cylinders and held at 35° F. The results will be found in table 5.

These data would not lead one to expect any marked rising of the fat in cream because of standardization. There was evidence of the fat moving upward somewhat faster at the beginning in the standardized cream, but at the end of 24 hours the differences between the top and bottom tests were practically the same. The results indicate that standardization is

TABLE 5

Butterfat content of various portions of standardized and unstandardized creams

TYPE OF CREAM	FRACTION	BUTTERFAT CONTENT AFTER			
		6 hours	12 hours	18 hours	24 hours
		per cent fat	*per cent fat*	*per cent fat*	*per cent fat*
Standardized	Top	36.0	36.0	36.25	36.0
	Middle	35.5	35.5	35.5	35.5
	Bottom	35.5	35.5	35.5	34.5
Not Standardized	Top	34.5	35.25	35.5	36.0
	Middle	34.5	34.75	34.5	34.5
	Bottom	34.5	34.5	34.5	34.0

almost a negligible factor in influencing the distribution of butterfat in cream.

Plasma Separation

The recent studies concerning the separation of plasma (usually referred to as "serum" or "milk layer") from bottled cream are more intimately connected with the problem of fat rising in cream. Trout and McCann (8), Garrison and Powell (3), and Mooney and Burgwald (5) have contributed to the knowledge concerning this problem. In addition, Doan (2) has made recommendations for reducing or controlling this separation. There was a general agreement among these investigators that high pasteurization temperatures reduce the plasma layer; that the less standardization required, the less plasma separation; that long storage periods favor the separation; and that high storage or creaming temperatures also favor the separation, although the distinctness of the plasma layer is decreased. However, there was a difference of opinion and in results obtained in regard to the effects of separation temperatures, agitation, heat treatment of the medium for standardization, and the time for standardizing.

During the preliminary trials for the fat rising studies, it was noticed that the cream upon standing formed a plasma layer at the bottom of the graduated cylinders. It was further noted that the plasma layer was not uniform or consistent in quantity or distinctness from one type of cream to another. Doubtless this observation has been made in practical creamery operation for whipping cream seldom shows a noticeable plasma layer. On the other hand it is frequently encountered in coffee creams and is considered quite objectionable. This accounts for the fact that the above investigators have all confined their studies to low fat creams. Inasmuch as it was convenient to make observations concerning this plasma layer, it was decided to estimate the quantity formed as closely as possible and record the results. These observations were made when the cylinders were

TABLE 6

Plasma separation occurring in creams held under different conditions

TYPE OF CREAM	SAMPLE NO.	EXTENT OF PLASMA SEPARATION AFTER			
		6 hrs.	12 hrs.	18 hrs.	24 hrs.
		ml.	*ml.*	*ml.*	*ml.*
High Fat Cream					
Raw held below 40° F.	1	+*	+	+	+
	2	0	+	0.25	0.5
	3	+	+	++	++
	4	0	0	+	+
	5	0	0	0	+
Pasteurized—held below 40° F.	1	+	+	++	++
	2	0	0	0	+
	3	0	0	+	+
	4	+	+	+	+
Raw—held at 54° F.	1	+	+	+	+
held at 84° F.	1	+	0.5	1.0	1.0
Medium Fat Cream					
Raw—held below 40° F.	1	0	0.25	0.5	1.0
	2	0	0.25	0.75	1.0
	3	0	0.25	0.75	1.0
	4	0	+	+	0.5
held at 54° F.	1	+	+	+	0.5
held at 83° F.	1	+	1.0	2.0	3.0
Pasteurized—held below 40° F.	1	+	0.25	0.5	0.75
	2	+	+	0.5	++
	3	0	+	1.0	1.0
Pasteurized milk—held below 40° F.	1	0	0	0	+
Low Fat Cream					
Raw—held below 40° F.	1	0.5	1.0	2.0	2.5
	2	0.5	1.0	1.75	2.0
	3	0.5	2.0	2.0	2.5
	4	1.0	1.5	2.0	2.0
	5	1.0	2.0	4.0	4.0
	6	+	1.0	1.5	2.0
held at 54° F.	1	0.5	1.0	4.0	3.5
held at 65° F.	1	2.0	2.0	4.0	5.0
Pasteurized—held below 40° F.	1	0.5	++	2.0	2.0
	2	+	++	2.0	2.0
	3	+	0.25	1.5	1.75
35% standardized raw held below 40° F.	1	+	+	+	0.5

\+ —trace.

++—less than .25 milliliters.

removed from the rack for the purpose of draining the cream. Table 6 includes the observations made on the plasma separation.

Of the factors studied, the fat content of the cream seemed to exert the greatest influence on the plasma separation. The separation in high fat cream was almost negligible, but the low fat cream always exhibited a distinct plasma layer after standing. The medium testing cream produced a plasma layer that was intermediate in depth between that in the high and low fat cream.

High holding temperatures increased the plasma layer to a very marked degree with all three types of cream. A standardization study was carried out only in one trial and the results did not deviate notably from the results that might have been expected with the other raw creams.

Pasteurization of the cream appeared to have a tendency to reduce the plasma separation slightly. The line of demarkation of the plasma layer was less distinct in the pasteurized cream. When the milk was pasteurized, and medium-fat cream separated from it, less plasma separation was evident.

As the length of the holding period increased, the plasma layer invariably increased in size. In the sample of medium-fat pasteurized cream held for three weeks the plasma layer was 22 milliliters and did not contain enough fat to be read in a cream test bottle. It suffices to say, that in general the observations on the plasma formation made in this experiment corroborate the results secured by the investigators mentioned previously.

SUMMARY AND CONCLUSIONS

1. The butterfat in cream has a tendency to rise and concentrate in the upper portion. This phenomenon is somewhat similar to cream rising in milk.
2. Fat rising in cream is decreased as the butterfat content is increased.
3. Low holding temperatures reduce the amount and rapidity of fat rising in cream.
4. Pasteurization of cream appears to increase slightly the extent of fat rising.
5. Standardization of cream with skimmilk does not appreciably affect the amount of fat rising.
6. Low fat contents and high holding temperatures tend to increase plasma separation in cream.

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THE RELATIVE EFFICIENCIES OF IRRADIATED ERGOSTEROL AND IRRADIATED YEAST FOR THE PRODUCTION OF VITAMIN-D MILK[1]

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It has been known for some time that the effectiveness of vitamin-D supplements depends upon the nature of the vitamin carrier. Hess, Weinstock, and Rivkin (1) showed that fewer rat units of vitamin D in the form of cod liver oil than in the form of viosterol were needed to protect infants against rickets. Hess and Lewis extended this observation to different kinds of vitamin-D milks which led to a differentiation between the rat unit value of an anti-rachitic and its clinical unit value (2). Numerous investigators (3) (4) (5) (6) (7) (8) have shown that the chick requires several times more rat units of vitamin D in the form of irradiated ergosterol than in the form of cod liver oil. Hess, Lewis, MacLeod, and Thomas (9) found this difference in effectiveness to apply to cows, 200,000 rat units of vitamin D in the form of irradiated ergosterol having been required per cow daily to produce milk having the same vitamin-D potency as that produced by cows fed 60,000 rat units daily in the form of irradiated yeast. Krauss, Bethke, and Monroe (10) further demonstrated that when irradiated ergosterol is fed to cows the vitamin D in the butterfat produced by these cows does not act in the same order of magnitude as does an equivalent amount of vitamin D in cod liver oil when fed to chicks.

Since irradiated ergosterol was at the time still being seriously considered as a means by which the vitamin-D content of milk might be increased through feeding, it was felt desirable to obtain further information as to the relative efficiencies of irradiated ergosterol and irradiated yeast for this purpose.

EXPERIMENTAL

Four groups of three Holstein cows each were fed according to the schedule in Table 1. In Experiment I the basal ration consisted of alfalfa hay of excellent quality, dried beet pulp, corn, oats, bran, and linseed oil-meal. In the second experiment alfalfa hay and corn silage furnished the

Received for publication May 28, 1934.

[1] Presented in part at the meetings of the American Dairy Science Association, at Urbana, Illinois, June 27, 1933.

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roughage part of the ration while the grain mixture remained the same as in experiment 1. Different cows were used in each experiment.

TABLE 1

Feeding Schedule

EXPERIMENT	DATE (1932)	GROUP I	GROUP II
I	Apr. 14–May 13	Basal	Basal
	May 13–June 6	Basal Plus 60,000 rat units Irradiated Ergosterol	Basal Plus 60,000 rat units Irradiated Yeast
II	Aug. 12–Sept. 12	Basal	Basal
	Sept. 12–Oct. 12	Basal Plus 120,000 rat units Irradiated Ergosterol	Basal Plus 120,000 rat units Irradiated Yeast

In Experiment I the irradiated ergosterol (corn oil solution) and the irradiated yeast were incorporated with the afternoon grain allowance, the amount of each required having been calculated from vitamin-D assays made in our own laboratory on these materials. In Experiment II the yeast and ergosterol were incorporated with the grain at each feeding (twice daily).[3]

During each of the last three days of each period indicated in Table I the entire milk production of each group was separated. The cream thus obtained was churned and the resulting butter rendered into pure fat. These fat samples were then stored in the dark in a cooler at 2° to 5° C. until assayed for vitamin D.

Blood samples were taken from the jugular vein of each cow one or two days before the close of a period, the samples from each group being combined immediately and dried over a steam bath.

On the last day of each period two defecations from each cow in each group were collected, combined, and dried in a hot air chamber.

The curative vitamin-D assays were made according to the standard "line test" procedure. The fat samples were melted and fed from a graduated pipette over a period of eight days. In some instances, particularly in the case of the samples from the control periods, the melted fat was incorporated in such amounts of the basal diet as were consumed in 7 to 8

[3] We are indebted to Acetol Products, Inc., New Brunswick, N. J., for furnishing the irradiated ergosterol, and to Standard Brands, Inc., New York City, for furnishing the irradiated yeast.

days. The dried blood and dried feces samples were incorporated in the basal diet in like manner.

In the one prophylactic trial the fat was incorporated in the basal diet and a daily record kept of individual food consumption. The rats were chloroformed at the end of five weeks. The radii and ulnae were removed and preserved in formalin until observed for degree of calcification after staining the sections with silver nitrate. The femurs were removed, cleaned of adhering tissue, dried and crushed, extracted with alcohol and ether, and then ashed.

RESULTS

The data showing the critical levels of butterfat, *i.e.*, the amounts required to produce definite healing, are presented in table 2. It should be pointed out that many other levels of fat were fed and that several hundred rats were used in arriving at these critical values.

TABLE 2

Potency of Fat (Curative)

	GROUP	SUPPLEMENT FED TO COWS	NO. OF RATS	CRITICAL LEVEL OF BUTTERFAT	RAT UNITS PER GRAM OF FAT	% FAT IN MILK	R. U. PER QT.
	I	None	13	6.0 gm.	0.17	3.4	5.7
	II	None	13	6.0 gm.	0.17	3.6	5.9
Exp. I	I	60,000 Irrad. Erg.	11	480 mg.	2.08	3.6	72.8
	II	60,000 Irrad. Yeast	11	320 mg.	3.13	3.7	112.7
	Ia	None	4	7.5 gm.	0.13	4.2	5.3
	IIa	None	5	6.0 gm.	0.17	3.8	6.3
Exp. II	Ia	120,000 Irrad. Erg.	29	500 mg.	2.00	4.3	84.0
	IIa	120,000 Irrad. Yeast	24	300 mg.	3.33	4.1	133.2

It is very apparent, from table 2, that when 60,000 or 120,000 rat units of vitamin D either in the form of irradiated ergosterol or irradiated yeast were fed the vitamin-D content of the butterfat produced was markedly increased and that the increase in vitamin-D potency was greater when the irradiated yeast was fed than when the irradiated ergosterol was fed. The ratio between the vitamin-D potency of the ergosterol fats and the yeast fats was in the order of 2 : 3.2. That this was not a chance result was indi-

cated by the agreement between the results obtained in the two experiments in which different cows were used.

The results obtained curatively were substantiated and emphasized prophylactically. In table 3 it will be noted that a diet containing 0.25

TABLE 3

Potency of Fat (Prophylactic)

SUPPLEMENT FED TO COWS	LEVEL OF FAT FED TO RATS	NO. OF RATS	AV. DAILY FOOD INTAKE	AV. TOTAL FAT INTAKE	CALCIFICATION*	BONE ASH
	Pct.		*Gm.*	*Mg.*		*Pct.*
120,000 Irrad. Erg.	0.25	6	7.7	674	0.25 +	29.98
120,000 Irrad. Yeast	0.25	6	7.8	683	1.17 +	37.06
120,000 Irrad. Erg.	0.50	6	6.9	1208	1.33 +	36.11
120,000 Irrad. Yeast	0.50	6	7.4	1295	2.58 +	40.24
120,000 Irrad. Erg.	0.75	6	7.3	1916	2.33 +	37.13
120,000 Irrad. Yeast	0.75	5	8.7	2284	3.20 +	46.42
Controls	.....	6	6.6	.....	0.0 +	25.90

* Width of Metaphysis: + = evidence of protection;
++ = fair protection;
+++ = good protection;
++++ = complete protection.

The numerical values were obtained by adding the plus signs of all rats in a group and dividing by the number of rats.

per cent of yeast fat resulted in as good calcification as was obtained with a diet containing 0.50 per cent of ergosterol fat and that 0.50 per cent of yeast fat was superior to 0.75 per cent of ergosterol fat. Some differences in total fat intake for the various groups will be noted. These were not large enough, however, to account for the difference in bone ash and calcification values. The greatest difference in total fat intake between two comparable groups (0.75 per cent level) was 368 milligrams. To obtain a difference of 1 per cent in the bone ash value of rats receiving, respectively, 0.50 per cent and 0.75 per cent of fat from the ergosterol-fed cows, it required 708 milligrams of fat; in the case of comparable groups receiving fat from yeast-fed cows it required 989 milligrams of fat to increase the bone ash value 6.18 per cent.

The close relationship known to exist between blood and milk prompted investigation of the vitamin-D content of the blood. It might be expected,

in view of the difference in vitamin-D potency of the two milks, that a difference would be found in the vitamin-D potency of the blood of the cows producing these milks. Such was not the case, according to the data shown in table 4. This would suggest a difference in absorption of vitamin

TABLE 4

Potency of blood (dry)

GROUP	SUPPLEMENT FED TO COWS	NO. OF RATS	CRITICAL LEVEL OF BLOOD	RAT UNITS PER GRAM OF BLOOD
I	None	15	500 mg.	2.0
II	None	15	500 mg.	2.0
I	60,000 Irrad. Erg.	5	175 mg.	5.7
II	60,000 Irrad. Yeast	6	200 mg.	5.0
Ia and IIa	None	8	300 mg.	3.3
Ia	120,000 Irrad. Erg.	9	150 mg.	6.7
IIa	120,000 Irrad. Yeast	9	150 mg.	6.7

D from the intestinal tract, yet no indication of this is apparent from the vitamin-D content of the feces (Table 5).

TABLE 5

Potency of feces (dry)

GROUP	SUPPLEMENT FED TO COWS	NO. OF RATS	CRITICAL LEVEL OF FECES	RAT UNITS PER GRAM OF FECES (DRY)
I	60,000 Irrad. Erg.	8	350 mg.	2.9
II	60,000 Irrad. Yeast	8	350 mg.	2.9
Ia and IIa	None	25	10.0 gm.	0.10
Ia	120,000 Irrad. Erg.	8	250 mg.	4.00
IIa	120,000 Irrad. Yeast	8	250 mg.	4.00

DISCUSSION

Some question may be raised regarding the failure of doubling the intake of vitamin D to bring about a greater increase in the vitamin-D content of the fat (Table 2). This failure can be partially explained on the basis of total fat production since, according to Hess, Light, Frey, and Gross (11), the lower the total production of butterfat the higher will be

the concentration of vitamin D in the fat. The average daily milk production of both groups of cows in both experiments was much the same but in Experiment I the fat production was less than in Experiment II (Table 6).

TABLE 6

Milk and fat production

PERIOD	GROUP	COW NO.	AV. DAILY MILK	% FAT	DAILY FAT
		Experiment I			
			Lb.		*Lb.*
	I	2	30.4	3.64	1.11
	Basal	380	34.0	3.00	1.02
		381	30.5	3.70	1.13
I				3.44*	
Apr. 14–May 13	II	1	33.0	3.41	1.13
	Basal	3	33.8	3.55	1.20
		382	32.4	3.86	1.25
				3.61	
	I	2	31.0	3.65	1.13
	Basal + 60,000	380	32.1	3.32	1.07
	units Irradiated	381	25.3	3.92	0.99
II	Ergosterol			3.61	
May 13–June 6	II	1	34.1	3.46	1.18
	Basal + 60,000	3	26.0	3.85	1.00
	units Irradiated	382	31.8	3.86	1.23
	Yeast			3.71	
		Experiment II			
	Ia	351	33.3	4.62	1.54
	Basal	373	32.3	4.44	1.43
		386	28.1	3.36	0.94
I				4.17	
Aug. 13–Sept. 12	IIa	352	42.9	3.80	1.63
	Basal	371	32.6	4.08	1.33
		384	25.5	3.28	0.84
				3.76	
	Ia	351	31.5	4.57	1.44
	Basal + 120,000	373	32.3	4.70	1.52
	units Irradiated	386	25.3	3.50	0.89
II	Ergosterol			4.32	
Sept. 12–Oct. 12	IIa	352	37.2	4.10	1.53
	Basal + 120,000	371	33.5	4.40	1.47
	units Irradiated	384	22.6	3.80	0.86
	Yeast			4.14	

* Total fat production of the group divided by total milk production of the group multiplied by 100.

In Experiment II it was intended originally to make the assays on cream. In order to preserve the cream 0.5 cc. of formaldehyde was added per quart. The assays on the cream did not prove satisfactory and after a period of two months the preserved cream was churned and rendered into fat. The critical levels given in table 2 are based on the assays made on fat so prepared. It was at first thought that the preservation of the cream with formalin may have destroyed some of the vitamin D. However, several samples of fat prepared from cream to which from 1.0 to 2.0 cc. of formalin per quart had been added, showed no lowered vitamin-D potency when compared with samples prepared identically from unpreserved cream. One of these samples was prepared from a cow receiving 120,000 rat units of vitamin D in the form of irradiated yeast and showed a potency of at least 200 units per quart. We are unable to explain the low potencies obtained in Experiment II but feel that the relationship shown between the two fats is valid. Thus the contention of Hess, *et al.* (9) that cows transfer the vitamin D of irradiated yeast to milk more efficiently than they do that from irradiated ergosterol is substantiated although the difference between the efficiencies is, according to our data, more nearly 3:2 than 3:1 as reported by Hess, *et al.*

According to Frey, Light, and Wilson (12) the rate of secretion of vitamin D into the milk is roughly proportional to the concentration of vitamin D in the blood. The rather small difference in concentration of vitamin D in the blood in Experiments I and II is in line with the potencies of the butterfats, as reported in table 2, in spite of the fact that in Experiment I the vitamin supplements were fed in the afternoon whereas in Experiment II they were fed both in the forenoon and afternoon. In both experiments the blood samples were drawn between 8 and 10 A.M.

In a previous paper (10) it was shown that only a small percentage of the vitamin D intake of cows is transferred to the milk. Other workers (11) (13) (14) have reported similar findings. From the data in table 5 it is apparent that a tremendous amount of vitamin D is excreted through the intestinal tract, but apparently there was no difference in the concentration of vitamin D in the feces of the ergosterol- and yeast-fed cows. This would eliminate any correlation between differences in absorption of vitamin D and efficiency of transfer to the milk.

It is quite possible that in the cows receiving irradiated ergosterol there was a greater storage of vitamin D in the liver and other tissues of the body. This would be in accordance with the recent work of Russell, Taylor, and Wilcox (15) whose results demonstrated that the failure of irradiated ergosterol to equal cod-liver oil in promoting bone formation in the chicken is not associated with any failure of the factor in ergosterol to enter certain tissues of the body. Also, McCoord and Luce-Clausen (16), in their study of vitamin A storage in the livers of rats, came to the conclusion

that the concentration of vitamin A in the blood is no indication of the amount that may be stored in the liver.

The possibility remains, as pointed out in the recent work of Bethke, Record, and Kennard (8) that species difference plays a large part in determining the efficiency with which a particular vitamin-D source is utilized. In their work it was shown conclusively that irradiated ergosterol and irradiated yeast were considerably less effective than the rat equivalent amount of cod liver oil for calcification in chicks. To attribute our results to the presence of different forms of vitamin D in irradiated ergosterol and irradiated yeast, both of which were equally effective in rats but not in cows, would be as safe a speculation as could be made with the data at hand. The data do suggest that the loss in efficiency of the irradiated ergosterol occurs in the transformation from blood to milk.

It is worthy of mention, in this connection, that some of our unpublished data indicate that the vitamin D in cod liver oil is somewhat more efficiently transferred to eggs than is that in irradiated ergosterol.

SUMMARY

Two groups of lactating Holstein cows were fed 60,000 rat units of vitamin D as irradiated ergosterol and 60,000 rat units of vitamin D as irradiated yeast daily. Two other groups of lactating Holstein cows were fed 120,000 rat units of vitamin D from the same sources daily.

Vitamin D assays of the milk produced by these groups of cows showed that the irradiated ergosterol was approximately two-thirds as efficient in allowing transfer of its vitamin D to the milk as was the irradiated yeast. No satisfactory explanation for this was found. It could not be attributed to a difference in absorption from the intestinal tract since the vitamin-D content of the feces and of the bloods was the same regardless of the supplement fed. The existence of different forms of vitamin D having different species effects is considered as a possible explanation, as is also a difference in the "disappearance" into the tissues of vitamin D from the two sources.

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ADDENDA

Since the above manuscript was submitted there appeared an article with an almost identical title (Russell, W. C., Wilcox, D. E., Waddell, J., and Wilson, L. T. The relative value of irradiated yeast and irradiated ergosterol in the production of vitamin D milk. JOUR. DAIRY SCI. 17: 445–453. 1934). These two articles are essentially in agreement except that in our work the difference in efficiency was demonstrated at the lower as well as the higher level of vitamin D intake.

THE EFFECT OF FEEDING RAW ROCK PHOSPHATE ON THE FLUORINE CONTENT OF THE ORGANS AND TISSUES OF DAIRY COWS

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The influence of fluorine ingestion upon the distribution and storage of fluorine in the animal body has been little studied. This is no doubt due to the fact that adequate quantitative methods for the estimation of fluorine have been wanting, or that available methods were extremely tedious and difficult to use. With the development of methods for the estimation of fluorine the problem becomes a practical one in the study of fluorine metabolism. The early investigations dealt with the distribution of fluorine in laboratory animals. Little work has been reported on the distribution and storage of fluorine in farm animals. This is of practical importance to man because of its relation to the quantities of fluorine present in meat products, particularly where feeds have been used which contain abnormally high quantities of fluorine.

A series of fluorine determinations were made on the tissues and organs of dairy cows fed various levels of raw rock phosphate containing 3.5 per cent fluorine. We wished to follow the distribution of fluorine in the tissues of the normal dairy cow; to determine in what tissues fluorine storage took place; and also to determine the influence of fluorine ingestion upon the normal distribution of fluorine in the tissues.

The presence of fluorine in the tissues of the animal body has been known for a long time. Knowledge concerning the fluorine content of normal animal tissues and the influence of fluorine ingestion upon the storage of fluorine in the body is quite meager. Gautier and Clausmann (7) have pointed out that fluorine was found by them in all tissues. These investigators, Bethke, Kick, Edgington and Wilder (2), Boissevain and Drea (3), as well as others have reported the fluorine content of teeth and bones. Bethke and coworkers reported approximately 0.0409 and 0.0231 per cent fluorine in the femurs of swine. Boissevain and Drea determined fluorine spectrographically in human teeth and reported 0.025 and 0.06 per cent

Received for publication June 25, 1934.

Published with the permission of the Director of the Wisconsin Agricultural Experiment Station.

These studies were supported in part through a fellowship granted by the Ruhm Phosphate and Chemical Company of Mount Pleasant, Tennessee.

fluorine in the enamel and dentine, respectively. Contamination of the drinking water with 2 parts of fluorine per million resulted in a significant increase in the quantity of fluorine in the enamel and dentine of the teeth. An increase in the fluorine content of the bones was found when fluorine was present in the drinking water. These investigators were unable to detect fluorine in other tissues or in milk. Brandl and Tappeiner (4), Sonntag (10), and Bethke *et al.* have also demonstrated that the feeding of fluorine increases its storage in the animal body, particularly in the bones. The fluorine content of the bones of adults has been shown to be greater than those of the young by Gautier and Clausmann (8). Wrampelmeyer (12) reported an increase in the fluorine content of adult teeth over that of the child. Gautier and Clausmann's results for dental enamel range from 0.118 to 0.180 per cent. These results are similar to those of Carnot (5) who reports 0.10 to 0.30 per cent fluorine in dental enamel. For other references the reader is referred to the reviews of DeEds (6) and McClure (9).

EXPERIMENTAL

The animals used for this study were Holstein dairy cows which had been placed on experiment as young heifers 4–6 months of age and continued thereon until after the third calving. Eighteen heifers were divided into 6 lots of three animals each. Lot I received a grain mixture, corn silage and alfalfa hay. Lot II received a grain mixture, corn silage and timothy hay. Lot III received timothy hay, corn silage and a grain mixture to which was added 2.50 per cent special steamed bone meal. Lots IV, V and VI received the basal ration of lot III with various levels of raw rock phosphate replacing a like quantity of the bone meal. Thus lot IV received 0.625 per cent rock phosphate, or 0.022 per cent fluorine, lot V received 1.25 per cent rock phosphate, or 0.044 per cent fluorine, and lot VI received 2.50 per cent rock phosphate, or 0.088 per cent fluorine. The same grain mixture was used throughout the experiment and the feeds were varied in amount to care for the needs of the growing animal and for lactation according to the Morrison feeding standards. Ground limestone was added to the rations of lots III to VI inclusive in order to maintain a comparable Ca : P ratio in all lots. The animals were removed individually from the experiment at the approximate age of five years. Only one animal from each of lots I to III was taken for analytical investigation, hence the control group represents 3 animals only.

Samples of teeth, bone, liver, kidney, heart muscle, pancreas, thyroid, hair and tendon were obtained. The teeth were divided into two sets. The incisor teeth were used for analyses of enamel and dentine. The outer enamel was carefully removed with a hand file, the tooth was then burnished on an emery stone to remove all traces of enamel and the remainder

of the tooth was used as representative dentine. All of the incisor teeth from each cow were thus used in making up a sample. The molar teeth from the right jaw were removed, the roots broken off and the crown of the tooth analyzed for its fluorine content. Due to the structure of cattle molars the amount of enamel present is not restricted to the outer layer of enamel but invaginations of enamel occur between the cusps of the tooth. Hence analyses of the molars should give values lying between enamel and dentine. The fat of the bones was extracted by hot alcohol. All tissues were dried in a drying room for 6 to 7 days. They were then removed, weighed, ground and stored in screw top sample bottles.

In the case of the hoof and hair scrubbing with a brush, soap and distilled water was resorted to in order to free them of contamination from excreta. The sample of hair was obtained from the tail. Skin and fat were obtained only in the case of normal animals. The skin was carefully shaved and washed free of adherent material while the fat free of connective tissue was saponified in order to make sure that the fluorine present, if any, could be held with Na_2CO_3 during the ashing process.

The method used in these analyses was that of Willard and Winter (11) as modified by Armstrong (1). Precautions were used to obtain proper ashing as we found that even small traces of carbon interfered with recovery of added fluorine. To obtain a white ash we adopted the following procedure. The sample was moistened thoroughly with enough Na_2CO_3 solution to incorporate from 1 to 4 gms. of Na_2CO_3, depending upon the size of the sample. The sample was then completely dried on a hot plate and ashed at a low temperature (about 650° C.) for not more than 20 minutes and usually only 10. The sample was then moistened with redistilled water, again dried and ashed. This was repeated until a white ash was obtained. From three to five washings were found to be necessary for most of the tissues.

The entire distillate was collected and titrated, using freshly prepared alizarin as an indicator. A determination of a blank containing all of the reagents was made at frequent intervals and the blank deducted from the titration of the samples. In our experience excellent results were obtained with this method when due precautions were observed with respect to ashing and titrating, and when small quantities requiring less than 10 cc. of $Th(NO_3)_4$ were used.

Analyses were made in duplicate for all but one cow in each lot. One cow was selected from each lot and quadruplicate samples were taken for cows No. 62, 66, 69, and 71. Two duplicate determinations were made without added fluorine while 0.532 mg. of fluorine was added to the remaining two. Thus the analyses of the tissues from one cow from each lot were completely checked by fluorine recoveries.

TABLE 1

The fluorine content of dairy cattle tissues expressed in mgs. per 100 gm. dry weight

	COW NO.	TEETH			BONE	LIVER	KIDNEY	HEART MUSCLE	PANCREAS	THYROID	TENDON	HAIR	HOOF
		Enamel	Dentine	Molar									
Controls—no added fluorine	57	22.50	44.90	52.90	60.05	0.55	0.69	0.23	0.69	.....	0.54	0.61	
	60	25.91	42.40	55.02	58.22	0.58	0.89	0.27	1.03	.....	.....	0.61	0.72
	62	31.57	99.45	53.35	56.92	0.52	1.01	0.27	0.85	0.68	0.75	0.70	
	Ave.	26.66	62.25	53.76	58.40	0.55	0.86	0.26	0.86	0.68	0.65	0.64	
Lot IV—added fluorine (0.022 per cent of the grain mixture)	64	291.08	399.60	381.02	544.92	0.78	3.18	0.48	0.70	68.66	.....	1.43	
	65	264.48	410.20	382.28	533.57	0.85	2.57	0.37	0.82	108.60	.....	1.17	
	66	290.42	477.00	392.92	527.82	0.53	2.55	0.88	0.95	.....	.....	1.34	
	Ave.	281.99	428.93	385.41	535.43	0.72	2.77	0.58	0.84	88.63	.....	1.31	
Lot V—added fluorine (0.044 per cent of the grain mixture)	67	301.72	531.20	476.53	635.36	0.73	3.47	0.75	0.83	.....	.....	0.93	0.82
	68	332.12	454.50	465.88	623.96	0.83	3.35	0.64	0.90	.....	.....	1.06	
	69	296.00	561.87	483.36	661.72	0.62	3.82	0.97	0.92	.....	.....	.....	
	Ave.	309.95	515.86	475.26	640.35	0.73	3.55	0.75	0.88	.....	.....	1.00	
Lot VI—added fluorine (0.088 per cent of the grain mixture)	70	685.52	777.50	708.32	945.44	0.85	4.30	0.94	0.91	164.20	1.12	1.39	
	71	690.70	865.38	709.84	938.35	0.77	4.37	0.87	1.06	.....	1.15	1.41	1.16
	72	688.56	839.50	726.56	965.20	.....	.....	.....	.....	.....	.....	.....	.....
	Ave.	688.29	827.46	714.87	949.60	0.81	4.34	0.91	0.98	164.20	1.14	1.40	

RESULTS

It is seen in table 1 that all of the tissues examined contained traces of fluorine. Bone contained on the average more fluorine than other tissues, although dentine was only slightly less. These structures including enamel apparently form the greatest place of storage of fluorine. The active organs such as the liver, heart muscle, pancreas, thyroid, kidney, tendon and hair were relatively free of fluorine as compared to bone for example. With the exception of the pancreas, and possibly the liver, each added increment of fluorine to the ration resulted in an increased storage of fluorine. This supports the work of Brandl and Tappeiner (4), Sonntag (10), Bethke *et al.* (2), and Boissevain and Drea (3).

In cattle the fluorine content of the dentine is higher than it is in the enamel. In this respect these analyses corroborate those of Boissevain and Drea. Their results show that the dentine contains nearly twice the amount of fluorine determined spectrographically as the enamel of human teeth. Our results obtained for cattle show 0.0266 per cent and 0.0625 per cent, respectively, for normal enamel and dentine. These values approximate those reported for human teeth by Boissevain and Drea. They are below the values reported for enamel by Gautier and Clausmann and by Carnot. The values obtained for normal bone lie within the range of fluorine content reported by Gautier and Clausmann. They are somewhat higher than the results obtained by Bethke *et al.* for swine.

It was impossible for us to demonstrate large amounts of fluorine in washed hair.

The results obtained in these studies show that additional fluorine in the normal ration increases the quantity of fluorine in the tissues. The most noteworthy increase in fluorine content was found in the bones, teeth, and thyroid. The latter increased its fluorine content 24 times on the high level of fluorine feeding. The amount of fluorine in the enamel increased 25 times over that of the normal when 0.088 per cent of fluorine was added to the grain ration. In the bones the increased quantity of fluorine was only 16 times that of the normal when 0.088 per cent fluorine was included in the grain mixture. The general relationship between the structures with respect to their fluorine content remained the same, namely, in the order of bone, dentine, and enamel. The remaining tissues with the exception of the pancreas and liver showed an increased fluorine content of a much

The results obtained by these studies suggest that fluorine storage closely follows the deposition of calcium and phosphorus. They also indicate that fluorine is not effectively excreted from the body. It is stored, perhaps as a means of removing it from circulation. It seems unlikely that sufficient fluorine can be stored or held in the meat to become a potential source of danger to the public health even when definitely chronic toxicosis has developed.

SUMMARY AND CONCLUSIONS

Fluorine was found to occur in all of the normal tissues studied. The greatest quantity of fluorine was found to accompany calcium and phosphorus deposition. Thus the bones and teeth contained large quantities of fluorine while the more active organs such as the liver, kidney, heart muscle, and the other tissues studied showed only small quantities of fluorine.

The quantity of fluorine present in normal dentine and normal bone of dairy cows was found to be similar and the average fluorine content was found to lie between 42 and 63 milligrams per 100 grams of dried tissue. The fluorine content of the liver, kidney, heart muscle, pancreas, thyroid, tendon, hair, and hoof was found to be less than 1 milligram per 100 grams of dried normal tissue. The inclusion of 0.088 per cent fluorine in the grain mixture resulted in an increased storage of fluorine in the bones and teeth. This amounted to 16 to 25 times that found in normal osseous structures. The internal organs, tendon, and hair likewise doubled in fluorine content when the grain mixture contained 0.088 per cent fluorine.

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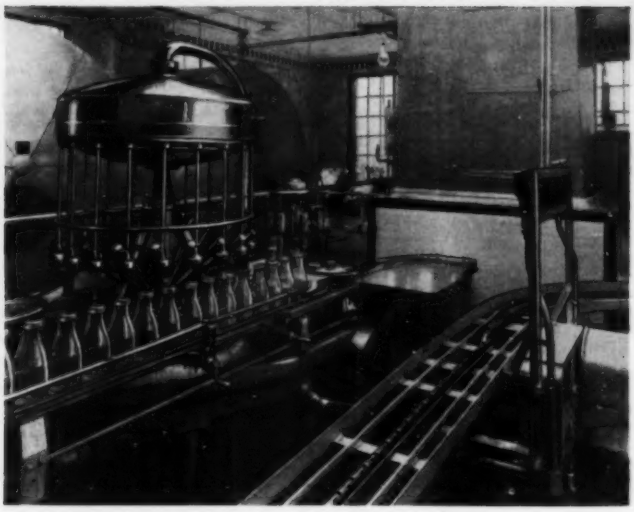

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Add the savings due to low upkeep to the many other savings possible with vacuum filling. The result is that the original cost of a Mojonnier Vacuum Filler is soon repaid. After that it continues to add substantial amounts to the profit side of the ledger.

Write for complete details, or better still, see the Mojonnier Vacuum Filler at the Dairy Industries Exposition in Cleveland in October.

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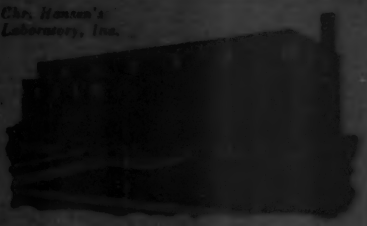

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